EXHIBIT 1

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231, on March 17, 1995

By _____

PATENT

Attorney Docket No. 014643-000310

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

BERNS et al.

Serial No.: 08/216,121

Filed: March 22, 1994

For: GENE TARGETING IN ANIMAL CELLS USING ISOGENIC DNA CONSTRUCTS

Examiner: B. Stanton

Art Unit: 1804

DECLARATION UNDER 37 CFR §1.132

Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

I, Dr. Anton Berns, declare as follows:

1. I am a co-inventor of the above-referenced application. I am employed as the Head of the Division of Molecular Genetics at The Netherlands Cancer Institute in Amsterdam, The Netherlands, a position I have held since 1985. In addition, since January 1995 I have served as the Laboratory Research Coordinator at The Netherlands Cancer Institute. I have a concurrent appointment as a Professor in Experimental Molecular Genetics of Inherited Diseases at the University of Amsterdam which I have held since 1992. From 1992 until 1994, I also acted as Executive Vice President of Research and Development for Somatix Gene Therapy Corporation in Alameda, California.

2. My education includes a Masters degree in chemistry from the University of Nijmegen, The Netherlands, and a Ph.D. in biochemistry received from the University of Nijmegen in 1972. A copy of my *Curriculum Vitae* is attached as Exhibit A.

3. I am familiar with the prosecution of the above-referenced patent application and have reviewed the examiner's comments in the rejection of the claims under 35 USC §112, first paragraph. As I understand the examiner's position, he believes that the method disclosed in the above-referenced application would not be successful for loci other than the retinoblastoma susceptibility (Rb) locus. However, as explained in Paragraphs 4-5, *infra*, work done in my laboratory and by other researchers demonstrates that the use of isogenic DNA vectors as taught in the subject specification results in increased targeting efficiency at loci other than Rb. This result would have been expected by a scientist reading the subject disclosure because there was no evidence that the Rb locus would behave anomalously (*i.e.*, differently from most genes) as a site of homologous recombination.

4. Subsequent to the filing of the subject application, other scientists carrying out gene targeting at a variety of genetic loci have found significant increases in the ratio of homologous to non-homologous recombination events result when isogenic, rather than non-isogenic, DNA vectors are used.

i) For example, van Deursen and Wiering, *Nucleic Acids Research* 20:15, 3815-3820 (1992), used homologous recombination to introduce site-specific mutations into the creatine kinase M (CKM) gene of mouse ES cells. These researchers found that when an isogenic targeting vector was used, homologous recombination occurred at high frequency (*i.e.*, 12%). In contrast, when a nonisogenic vector was used no homologous recombination events were found (*i.e.*, a frequency of 0%). This paper clearly demonstrates that the use of isogenic DNA targeting constructs increases the ratio of homologous to non-homologous recombination events.

ii) As another example, Deng and Capecchi, *Molecular and Cellular Biology* 12:8, 3365-3371 (1992), carried out experiments using the HPRT locus in mouse ES cells. These authors reported that vectors prepared from isogenic DNA targeted four to five times more frequently than corresponding vectors from nonisogenic DNA, demonstrating an increase in correct gene targeting at the HPRT locus by use of isogenic DNA vectors.

iii) As another example, Deng *et al.*, *Molecular and Cellular Biology* 13:4, 2134-2140 (1993), used a replacement vector containing isogenic DNA to introduce a mutation into the mouse cystic fibrosis transmembrane regulator (CFTR) gene in order to create a mouse model for human cystic fibrosis. Deng *et al.* noted that the targeting frequency

achieved by them was significantly higher than that reported by other investigators (*i.e.*, Koller *et al.*, reference 10 of Deng *et al.*) who used a similar replacement type vector containing non-isogenic DNA (*see* Deng *et al.* at page 2139, column 1, lines 15-20). In hypothesizing that this difference in targeting frequency may be due to the use of an isogenic DNA vector, Deng *et al.* cited the publication by myself and my coinventors disclosing the subject invention (te Riele *et al.*, 1992, *Proc. Natl. Acad. Sci. (USA)* 89:5128-5132). Copies of the van Deursen and Wiering, Deng and Capecchi, Deng *et al.* and Koller *et al.* references are enclosed with the accompanying Form PTO-1449.

iv) The results reported in the three references described *supra* demonstrate that the use of isogenic DNA vectors targeted to a variety of genes results in significant increases in the ratio of homologous to non-homologous recombination, as taught by the subject application. The method taught in the subject application is clearly not limited to any particular gene or locus.

5. In addition, work done by me or under my supervision has demonstrated that following the teachings of the subject application, use of isogenic targeting vectors results in high ratios of homologous to non-homologous recombinants at several loci in addition to the Rb locus. The results of several experiments carried out using mouse ES cells are summarized in Table 1, *infra*. These experiments do not represent direct side-by-side comparisons using targeting vectors with isogenic and nonisogenic DNA. However, based on my knowledge of the literature in this field, the high targeting efficiency observed indicates that using isogenic DNA vectors results in an increased ratio of homologous to non-homologous recombination at each of the loci tested. The experiments that generated the results for three of the loci listed in Table 1 have been described in scientific publications, copies of which are enclosed with the accompanying Form PTO-1449.

Table I

Locus	% Correct Gene Targeting
mdrla[1]	10%
bmi-1[2]	14%
IL2Rγ	30%
bcl-3	7%
NF-2	40%
E12[3]	40%
Frat-1	12%
P107 (Rb related gene)	30%
rep3	3%
CD44	10%
integrin-β4	9%

[1] Schinkel *et al.*, *Cell* 77:491-502, (1994)
[2] van der Lugt *et al.*, *Genes & Development*: 8:757-769 (1994)
[3] Bain *et al.*, *Cell* 79:885-892 (1994)

6. The results in Table 1, *supra*, together with the results reported by other researchers using isogenic targeting vectors as described in Paragraph 4, also demonstrate that the method of the subject invention is useful for producing populations of cells where between about 10% and 90% of the cells exhibit correct gene targeting.

7. As I understand the examiner's position, he believes that the method disclosed in the above-referenced application would not work for cells other than mouse ES cells. I am not aware of, nor do I find in the examiner's comments in the Office Action, any

scientific reason to support this assertion. I know of no data suggesting that the fundamental systems dealing with extrachromosomal recombination in ES cells is unique or different from that generally found in other somatic cells. On the contrary, a scientist would expect ES cells and other somatic cells to have similar properties. For example, Charron *et al.* *Molecular and Cellular Biology* 10:4, 1799-1804 (1990) described experiments using the vector pJC7, encoding n-myc and using a neomycin resistance/promoter selection system, for gene targeting in ES cells and in pre-B cell lines. Charron *et al.* reported that the targeting frequency in two pre-B cells lines was comparable to the frequency in ES cells (*see* Table 2 of Charron *et al.* showing targeting frequencies of 17% and 22% in pre-B cells, and frequencies of 0%, 0%, 44.4%, 100%, 25%, 16.7% and 26.7% in ES cells). The results reported by Charron *et al.* are consistent with the view held by scientists that there is no reason to believe that the fundamental aspects of extrachromosomal recombination differ between mouse ES cells and other mammalian cells. The Charron *et al.* reference is enclosed with the accompanying Form PTO-1449.

8. I have reviewed the examiner's comments in the rejection of claims under 35 USC §103. As I understand the examiner's position, he understands the comments of Capecchi, *Science* 244:1288-1292 (1989), and Sedivy and Sharp, *Proc. Natl. Acad. Sci. (USA)* 86:227-231 (1989), regarding the "extent of homology" to refer to the level of sequence identity between input and chromosomal sequences. However, a scientist reading these references would understand that these authors are referring to the *length* of the regions of homologous DNA and not to the degree of homology (*i.e.*, degree of sequence identity). For example, the Capecchi reference at page 1289, column 2, lines 11-17 clearly indicates that the "extent of sequence homology" can be described as ranging (in the experiments discussed) "from 2.9 to 14.3 kb." This is clearly a description of length and not sequence identity.

9. Similarly, in the Sedivy reference "extent of homology" is plainly used to refer to *length*, as shown at page 230, column 2, last paragraph, lines 8-11. See also page 231, bridging sentence, referring to "larger" (*i.e.*, longer) homologous sequences. The sentence in the Sedivy reference particularly cited by the examiner for teaching "maximization of

homology" (Sedivy *et al.*, page 227 first column, first paragraph, lines 15-21) does not refer to work by Sedivy but to Sedivy's references 3 and 5. Sedivy's reference 3 (Lin *et al.*, 1985, *Proc. Natl. Acad. Sci. (USA)* 82:1391-5) and reference 5 (Thomas and Capecchi, 1987, *Cell* 51:503-12) are enclosed with the accompanying Form PTO-1449. Neither of these references discusses or teaches maximization of sequence identity or the advantages of using isogenic DNA constructs. The paper by Thomas and Capecchi includes only experiments in which the *length* of the homologous region between target and vector is varied (*see, e.g.*, Table 3 of that paper). Similarly, Lin *et al.* describe the deletion of regions of the targeting vector, and not differences in sequence identity.

10. As I understand the examiner's position, the examiner also maintains that one of skill reading Waldman *et al.* (1988) would appreciate the importance of the degree of sequence identity in gene targeting as disclosed in the subject application. However, Waldman *et al.* do not teach gene targeting but instead, describe *intra*molecular recombination. A scientist would not have extrapolated Waldman's results with intramolecular targeting to the design of a gene targeting vector for a number of reasons, some of which are listed below.

i) Intramolecular targeting differs from gene targeting (*i.e.* integration of gene targeting vectors by extrachromosomal recombination) in a number of ways. For example, it was known that these processes are effected by different mechanisms (*see* Klein, 1984, *Nature* 310: 748-753) and have fundamentally different cellular consequences. Intrachromosomal recombination between related chromosomal sequences is generally harmful to a cell and must be suppressed. By contrast, extrachromosomal recombination may have an essential role in cellular physiology in effecting repair of double-stranded DNA breaks and may therefore need to be facilitated. These different physiological roles would have suggested that stricter sequences requirements would apply for intrachromosomal than extrachromosomal recombination so that latter occurs with greater efficiency than the former.

ii) An earlier paper by Waldman and Liskay, *Proc. Natl. Acad. Sci. (USA)* 84: 5340-5344 (1987), indicated that the degree of sequence identity required for intramolecular recombination was greater that required for intermolecular recombination. These authors studied the effects of a 19% base-pair mismatch on genetic recombination and found that intrachromosomal recombination was reduced by a factor of greater than 1000, while

extrachromosomal recombination was reduced only 3- to 15-fold. The authors noted that "Our results suggest differences between the mechanisms of extrachromosomal and intrachromosomal recombination in mammalian cells." In view of this manifest difference in sequence specificity of intra- and extrachromosomal recombination a scientist would not have expected the Waldman (1988) publication regarding intrachromosomal recombination to have applied to gene targeting (*i.e.*, extrachromosomal) systems.

iii) The experiments described by Waldman on intracellular recombination were performed on segments of DNA much smaller (*i.e.*, 360 bp) than those typically used in gene targeting (several kb). Waldman states that "efficient [intramolecular] recombination appears to require between 134 and 232 bp of uninterrupted homology" (abstract). In view of the teaching of Capecchi and others of the importance of the length of targeting DNA in obtaining high efficiency in gene targeting, the purported identification of a targeting unit in the context of a DNA segment having a length of less than 232 bp would have been of little predictive value in the context of the much larger targeting DNA segments used in gene targeting.

iv) For these and other reasons a scientist studying *gene targeting* would not have understood the work by Waldman *et al.* on *intramolecular* recombination to suggest that isogenic DNA vectors would result in considerable increases in the frequency of homologous recombination.

11. The examiner contends that prior to the disclosure of the subject invention, a scientist would have been motivated to use an isogenic targeting vector to optimize targeting efficiency "in any situation in which targeting efficiency was low." However, prior to the disclosure of the instant invention numerous scientific publications described efforts to increase targeting efficiency. A variety of approaches were suggested by others, including increasing the length of the homologous DNA, development of the PNS (positive-negative selection) system, and use of constructs lacking an essential element (*i.e.*, promoters, translation initiation or poylyadenylation sites) that were recovered upon recombination. However, no publication of which I am aware suggested that using isogenic DNA would result in a dramatically increased frequency of homologous recombination as disclosed in the subject application.

12. Furthermore, in the absence of a compelling motivation such as that provided by the subject invention, a scientist would not have taken the special steps required to use isogenic DNA vectors for gene targeting. A scientist, intending to target a particular gene, would typically use a homologous clone that was easily available. Prior to the disclosure by myself and my co-inventors, the advantage of an extremely high (*e.g.*, greater than about 99.5%) level of sequence identity was not understood. In the absence of this understanding scientists did not go to the considerable time, effort and expense of using isogenic DNA constructs. Following the discovery of the advantages of using isogenic DNA vectors by myself and my coinventors, the practice of scientists in the field changed. For example, a large proportion of the research carried using gene targeting is carried out using mouse ES cells. Most mouse ES cells are derived from the 129 strain of mouse. In contrast, at the time of invention, most of the mouse genomic libraries used for gene targeting in these ES cells were derived from the BALB/c or Black 6 mouse strains. Prior to our discovery of the advantages of isogenic DNA vectors, experts in the field believed that because the genomic DNA contained in the 129 ES cells and the vector DNA found in BALB/c and Black 6 (BL6) genomic DNA libraries were homologous (*i.e*, they were both from mouse) that these cells and vectors were well matched for gene targeting studies.

13. However, following our discovery, workers in the field became aware of the advantages of using isogenic DNA and have, in many cases, modified their protocols to use isogenic DNA vectors. Since disclosure of the advantages of using isogenic targeting vectors, researcher in numerous laboratories around the world have contacted my laboratory with requests for aliquots of a genomic library made from mouse strain 129 cell DNA to use in conjunction with their strain 129 ES cells. I have attached as Exhibits B-P letters from investigators requesting aliquots of the strain 129 genomic library. These requests demonstrate acknowledgement by members of the scientific community of the advantages of using isogenic DNA vectors. They also demonstrate that researchers targeting 129 ES cells had not, prior to the disclosure of our invention, been motivated to use isogenic DNA for constructing gene targeting vectors. I estimate that I have received more than twenty-five such requests.

14. As I understand the examiner's comments at page 11 of the Office Action, he suggests that it would have been obvious to one of skill to use isogenic DNA constructs to increase the efficiency of gene targeting. However, in my opinion as an expert in the field, the position set forth by the examiner include reasoning that is contrary to accepted scientific belief or, at best, unsupported by experimental evidence.

15. For example, the examiner's argument appears to rest on the theory that homologous recombination is dependent on nucleation between the vector DNA and the genomic target DNA. Nucleation is described as "an essentially unimolecular collision reaction" that is a critical, rate-limiting step in the homologous recombination reaction. In my opinion the examiner misconceives the process of homologous recombination and the conditions under which recombination occurs. Homologous recombination involves cellular machinery such as recombinases, endonucleases, repair enzymes, DNA binding proteins, and possibly such incompletely characterized processes as strand invasion and long patch mismatch repair. Homologous recombination is simply not comparable to collision reactions occurring between simple molecules in solution. Notably, if recombination occurs as a unimolecular collision, as described by the examiner, one would expect that the rate of recombination would depend on the concentration of vector DNA introduced into the cell. However, there is no evidence to support this notion.

16. The examiner also suggests that the results disclosed the subject application could be accounted for by fortuitous presence of "particular 5 base pair region[s]" that are "critical" to the nucleation reaction (Office Action at page 11, lines 19-21). However, experiments carried out by me or under my supervision indicate that particular "recombinogenic" sequences do not account for the surprising results obtained using isogenic DNA vectors. We constructed two vectors comprising corresponding regions from the mouse Rb locus. One vector was made using DNA from strain 129 mice; the second was made using DNA from Balb/c mice. Gene targeting experiments using ES cells derived from 129 or BALB/c strain mice were carried out essentially as described in the specification. The results are summarized in Table II. If there were, *e.g.*, a recombinogenic stretch in, for example, the 129 strain DNA it would be expected that using this DNA would result in a higher frequency of homologous recombination without regard to the strain of ES cells used.

However, targeting efficiency was considerably greater (by more than an order of magnitude) when either DNA vector was used with ES cells from the corresponding strain, compared to either DNA vector used with ES cells of a different strain. These results demonstrate that it is the *isogenicity* of the target DNA and the targeting vector that results in higher targeting efficiency, not any *particular* sequence present.

Table II

Mouse strain from which vector DNA is derived	Source of ES cells	% Correct Gene Targeting (homologous recombination/non-homologous recombination)
129 strain	129 strain	26% (33/94)
129 strain	BALB/c strain	0.6% (1/144)
BALB/c strain	129 strain	1.4% (1/68)
BALB/c strain	BALB/c strain	18% (16/72)

I have been duly warned that willful false statements and the like are punishable by fine and imprisonment or both under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-identified patent application or any patent issuing thereon.

Respectfully submitted,

Dated: March 14, 1995

Anton Berns, Ph.D.

CURRICULUM VITAE

Name	Berns, Anton J.M.
Born	January 3, 1945 Schijndel, The Netherlands
Education	
1957 - 1963	Gymnasium-ß, Canisius College, Nijmegen
1963 - 1969	Masters degree Chemistry (with honors) in Biochemistry, Organic Chemistry and Physical Chemistry University of Nijmegen, The Netherlands
1969 - 1972	Ph.D. study (Supervisor Prof.Dr. H. Bloemendal) Thesis (with honors): Isolation of calf lens mRNA and its translation in heterologous systems University of Nijmegen.
1972	2-months visit Massachusetts Institute of Technology (Dept. Drs. A. Rich and D. Baltimore)
1972 - 1974	Junior staff member Department of Biochemistry, University of Nijmegen, The Netherlands
1974 - 1976	Postdoc Salk Institute, San Diego, California
1975	Animal Virology Course, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York
1976 - 1985	Senior staff member Dept. of Biochemistry, University of Nijmegen, The Netherlands
1979	6 months visiting scientist Salk Institute
1985 - present	Head Div. Molecular Genetics, The Netherlands Cancer Institute, Amsterdam, The Netherlands
1992 - present	Professor in Experimental Molecular Genetics of Inherited Diseases, University of Amsterdam
1992 - 1994	Executive Vice President R&D of Somatix Gene Therapy Corporation, Alameda. Cal.
1995 -	Laboratory Research Coordinator, Netherlands Cancer Institute.
Honors	
1972	Travel stipend from SHELL
1973	Gold medal award of the Chemical Society, The Netherlands
1993	Biology Prize " Antoine de Lacassagne" of The French Cancer Society
Memberships etc.:	
1989 - present	Member "Scientific Board of the Dutch Cancer Society"
1990 - present	Member EMBO
1989 - 1991	Chairman Genetic Society
1989 - present	Member committee "Genetics and Virology", NWO
1992 - present	Chairman committee "Genetics and Virology", NWO Groupleader Working groups NWO: SON (nucleic acids), SLW (Molecular developmental biology of animals, GMW (persistent virus infections and oncogenic transformation)
1991-1994	Co-organizer Mouse Molecular Genetics Meetings (Cold Spring Harbor/Heidelberg)
Editorial Boards:	
1991-	BBA, Reviews in Cancer
1995-98	EMBO J.
1995-	Genes & Development

Accepted invited lectures from July 1992

- Parijs, Institut Pasteur. Seminar 2-3 juli 1992. Multistep tumorigenesis: Effects of gain- and loss-of-function mutations in oncogenes and tumor suppressor genes in transgenic mice.

- London, Wellcome Summer School on Gene targeting and homologous recombination. 9-18 juli 1992. Targeted disruption of the pim-1 oncogene and the retinoblastoma tumor suppressor gene.

- Marburg, 3rd IMT Symposium. 5-7 oktober 1992:
Multistep transformation: tumor induction in mice with gain-of-function and loss-of-function mutations in oncogenes and tumor suppressor genes.

- Cape Cod, AACR "Normal and Neoplastic Growth and Development", 18-22 oktober 1992. Tumor induction in mice with gain- and and loss-of-function mutations in oncogenes and tumor suppressor genes.

- Titisee, Somatic Gene Therapy- Gene transfer and Differentiation. 4-8 November 1992. Gain- and loss-of-function mutations in mice to identify new oncogenes and to determine their mechanism of action.

- Köln, Ernst Klenk Conference on "Regulation of Cell Growth". 8-10 november 1992. Oncogenes and growth factors.

- Lausanne 2nd ISREC Conference, 14-15 januari 1993. Genetic damage and escape from proliferation control. Multistep transformation in mouse model systems.

- Big Sky. AACR 1-5 februari, 1993. "Oncogenes and antioncogenes in differentiation, development and human cancer. Identification and characterization of synergizing oncogenes.

- Salk Institute. La Jolla. Seminar. Mouse model systems to study oncogenes and tumor suppressor genes.

- Brussel. EACR 7 april. Identification of collaborating oncogenes in lymphomagenesis: effects of gain- and loss of function.

- IMP Seminar, 8 april 1993. Identification of collaborating oncogenes in lymphomagenesis: effects of gain- and loss of function.

- University of Pittsburg. Seminar. Mouse model systems to identify and characterize synergizing oncogenes and tumor suppressor genes.

- Los Angeles. AMGEN. Seminar. Mouse model systems to identify and characterize synergizing oncogenes and tumor suppressor genes. Round table discussion about Somatix Gene Therapy programs

- Copper Mountain. FASEB Meeting on Cellular and Molecular Genetics. Juli 11-16, 1993. Identification and characterization of synergizing oncogenes.

- Stanford, Beckman Institute, Seminar. Identification and characterization of synergizing oncogenes.

- San Francisco, CHI conferences. 22-23 september, 1993. Identification and characterization of synergizing oncogenes.

- Heidelberg. Gene Diagnosis and Gene Therapy. 4-6 oktober, 1993. Tumor induction in mice with gain- and and loss-of-function mutations in oncogenes and tumor suppressor genes.

- Seattle, Hutchinson Cancer Center. 21 oktober, 1993. Mouse model systems to identifiy and characterize synergizing oncogenes.

- Strassbourg, Human Frontier Science Program Symposium. 19 november 1993. Final report on collaboration with S. Tonegawa and M. Hooper on T cell receptor mutant mice (transgenics/K.O.).

- Ein Gedi, Israel. february 28 - March 4, 1994. Gene Therapy Conference. Immunotherapy using tumor vaccines transduced with GM-CSF

- UCSF. Seminar. Identification and characterization of synergizing oncogenes

- Noordwijk, April 23-26, 1994. The Netherlands. 4th European workshop on cytogenetics and molecular genetics of human solid tumors. Lecture: Mouse models to identify genes involved in initiation and progression of tumorigenesis.

- Vienna. May 23-25, 1994. IMP Conference " Molecular mechanisms of human disease". Mouse model systems to study multistep tumorigenesis

- Cold Spring Harbor. June 1-8. Symposium. Molecular Genetics of Cancer. Mouse model systems to study multistep tumorigenesis.

- Bar Harbor. August 29, 1994. Jackson Laboratory. Mouse model systems to identify and characterize collaborating oncogenes.

- Cold Spring Harber. September 1-5, 1994. Mouse Molecular Genetics Meeting. Co-organizer (together with Andy McMahon, Robb Krumlauf and Liz Robertson).

- Amsterdam, September 6-10, 1994. EORTC Breast Cancer working conference. Gene therapy approaches to treat cancer. A Sisiphian task?

- New Delhi, September 19-22, 199416th International Congress of Biochemistry and Molecular Biology. Mouse model systems to study the multistep process of tumorigenesis.

- Fulgsocentret, Denmark. October 20., 1994. Key note address. Danish Biochemistry Society. Mouse model systems to study the multistep process of tumorigenesis.

- Ein Gedi Israel. November 28 - December 2, 1994. 9th Maimonides Conference. Genes collaborating with myc in tumorigenesis.

- Keystone, Oncogenes, 20 years later. Jan 5-11, 1995. Identification and characterization of collaborating oncogenes in genetically manipulated mice.

- Zürich, March 15-17 1995. Meeting of Charles Rodolphe Brupbacher Foundation. Genetic predisposition to Cancer.

- Madrid, April 24-28. Nuclear oncogenes and transcription factors in hematopoietic cells. Identification and characterization of synergizing oncogenes in lymphomagenesis.

- Mosbach, Germany. April 27-29. Mosbacher Colloquium. The biochemistry and molecular biology of tumor development. Basic science at the doorstep of clinical medicine.

- Vienna, May 11-13, 1995. IMP Conference. Interfaces between Cancer and Development.

- San Francisco, July 23-29, 1995. Leukemogenesis and proto-oncogenes. Immunology Congress.

- Heidelberg, August 23-27, 1995. Mouse Molecular Genetics Meeting.

Grant support last 5 years

Program Grant NWO 1988-1993. Targeted disruption of genes. fl 1.500,000

Program grant NWO 1994-1999. Generation of mutant mouse model systems fl 1.400.000

Human Frontier Science Program Grant (With S. Tonegawa and M. Hooper) $ 750,000

STW/pharmaceutical Industries 1986-1992. fl 1.500,000

Dutch Cancer Society (NKB) : NKI 88-03, 1988-1992, fl 600,000
: NKI 89-17, 1989-1993, fl 600,000
: NKI 90-11, 1990-1994, fl 800,000
: NKI 90-12, 1990-1994, fl 600,000
: NKI 92-48, 1992-1996, fl 500,000
: NKI 94-771, 1994-1998, fl 900,000

Publications

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Favre, A., U. Bertazzoni, A.J.M. Berns and H. Bloemendal. A poly A content and secondary structure of the 14S calf lens messenger RNA. Biochem. Biophys. Res. Comm. 56: 273-280, 1974.

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LUNDS UNIVERSITET
Institutionen för Medicinsk och Fysiologisk kemi

UNIVERSITY OF LUND
Department of Medical and Physiological Chemistry

Dr. Anton Berns
Head Section Molecular Genetics
The Netherlands Cancer Institute
Plesmanlaan 121
1066 CX Amsterdam
The Netherlands

Lund October 21, 1991

Dear Dr. Berns,

I do recall that we met last october in Freiburg in Rolf Kemlers laboratory. I was there as a participant in an EMBO course of embryonic stem technology that was headed by Rolf Kemler. I also remember that you gave a very interesting lecture about PIM-1oncogene during lymphomatosis and in normal development. I tried to contact you over the phone last friday but you were not there so talked to one of your collaborators whose name I did not wrote down. Therefore I hereby ask you if it is possible for me to get some of your very good genomic library made from 129 mouse in addition to an ES cell line called E14 which primarily comes from Hooper. A fiend of mine, Dr. Björn Vennström at Karolinska Institutet, Stockholm, told me that he very kindly got those items from your laboratory. I am going to do homologous recombination with a gene coding a 62 kDa liver protein which we suspect probably effects the skeletal development during the embryogenesis in mouse. Since most of the ES cells available are cloned from 129 mice I would like to isolate the gene from an isogenic genomic library to be able to increase the events of homologous recombination.

Sincerely yours

Ahndera Franzen
Ass. professor

Department of Physiological Chemistry
P.O. Box 94
S-221 00 LUND
SWEDEN

Tel.no: 46 46 108578
Fax.no: 46 46 113417

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EXHIBIT C

MAX-PLANCK-INSTITUT FÜR PSYCHIATRIE
DEUTSCHE FORSCHUNGSANSTALT FÜR PSYCHIATRIE
AM KLOPFERSPITZ 19A
8033 PLANEGG-MARTINSRIED

Max-Planck-Institut für Psychiatrie
Am Klopferspitz 19a 8033 Planegg-Martinsried

TELEFON (089) 85781
DURCHWAHL 8578 / 3648
TELEX 521740 mpipsy

23.09.91

Dear Dr. Riole,

We are writing to you concerning the interesting results you presented at at a meeting in Heidelberg recently, namely, that in gene targetting experiments, targetting constructs made from syngenic DNA are more effective than those made from isogenic DNA. We have started to use gene targetting methodology as a tool in our studies of the nervous system and in order to optimise the system we would extremely eager to use constructs made from isogenic genomic clones.

Therefore, we would be very grateful if you could send us an aliquot of the genomic bank from the mouse strain 129. The library would not be distributed outside of this Dept. without your permission and we would be happy to share our results with you.

Looking forward to hearing from you soon,

Yours sincerely,

Prof. Hans Thoenen.

Hans Thoenen

Dr. Yasuo Masu.

Yasuo Masu

Dr. Patrick Carroll.

Patrick Carroll

FAX NO. 49-89-8578-3749

ZÜRICH UNIVERSITY MEDICAL SCHOOL
Institute of Physiology

Facsimile Transmission of ...1... page(s) incl. this page

Date: 29.12.92

To: Dr. A. Berns
Dr. H. te Riele
Div. of Molecular Genetics

Fax: 0031 20 512 ~~1998~~ 80 11

From
Dr. Max Gassmann, DVM
Institute of Physiology
University of Zürich-Irchel
Winterthurerstr. 190
CH-8057 Zürich
SWITZERLAND

Tel. +41 1 257 5051
Fax +41 1 364 0564

Dear Dr. Berns
Dear Dr. te Riele

I am writing to request the 129-derived genomic DNA library used for your Rb gene targeting experiments published in PNAS (June 92).

I am presently working at the University of Zürich after leaving Paul Berg's lab at Stanford 3 months ago. My goal is to continue my postdoctoral work on the characterization of a polyoma-based vector which replicates autonomously in ES cells(a poster was presented this summer at the CSH mouse meeting). Since such a vector might increase the frequency of homologous recombination I would like to do some targeting experiments using isogenic DNA. My lab is mainly interested in the regulation of the erythropoietin gene expression.

Since I will also join the meeting "Progress in Cancer Research" to be held at Lausanne (Switzerland) next month, I would be delighted if you could bring the library with you. Please be assured that we will mention you in any publication concerning this work. I am looking forward to see you soon. Thank you very much for your time and consideration. I wish you a happy New Year.

Sincerely,

M. Gassmann

cc. Hofer 30/12 Berns 13/1/93

M A X - P L A N C K - G E S E L L S C H A F T Z U R
F Ö R D E R U N G D E R W I S S E N S C H A F T E N E.V.

Arbeitsgruppe "Zellteilungsregulation & Gensubstitution"

H u m b o l d t U n i v e r s i t ä t

Postanschrift:

Max-Delbrück-Haus
R.-Rössle-Str. 10
O-1115 Berlin-Buch

Tel.: +49 30/9463307
FAX: +49 30/9463306

Dr. Anton Berns
Division of Molec. Genetics
Netherlands Cancer Institute
and Dept. Biochem. University
Plesmanlaan 121
1066 CX Amsterdam
Niederlande

Berlin, d. 27.7.92

Hein, Jansen

Dear Dr. Berns,

I like to congratulate you and your coworkers to the outstanding results you have recently published in P.N.A.S. I think this is a great breakthrough in homologous recombination.

I would be very interested to use your approach to inactivate the Rb gene in differentiated cell types like epithelial cells. We have recently published a paper in Oncogene describing the inactivation of pRb synthesis by antisense oligonucleotides which led to stimulation of cell division. In the mean time we can do this even better with antisense constructions and ribozymes. With your efficiencies of homol. recombination it is obviously possibly to achieve the knockout of both allels successively or even at the same time.

We are particularly interested in the knockout of the Rb gene in hepatocytes for several reasons. I would like to ask you if you are willing to collaborate on this matter. I can think of two alternatives. One would be to send one of my coworkers to your lab who is experienced in all essential techniques. The other alternative would be if you provide us with your targeting vectors and we try it on our own. In case of success, the results could be published together. I would be very pleased if you would be interested to collaborate on this matter.

Looking forward to your answer.
Yours sincerely,

Michael Strauss

Michael Strauss, Ph.D.

Beantwoord op 8/07/92

MAX - DELBRÜCK - LABORATORIUM
IN DER MAX-PLANCK-GESELLSCHAFT

Carl-von-Linné-Weg 10, D - 5000 Köln 30, Tel.: 0221-5062 620 FAX: 0221-5062 613

Dr. Carmen Birchmeier

Dr. Anton Berns
The Netherlands Cancer Institute
Division of Molecular Genetics
Plesmanlaan 121
1066 CX Amsterdam
The Netherlands

5.11.1991

Dear Dr. Berns,

For our further knock-out experiments, we want to use genomic clones from an 129 Sv library and I would therefore appreciate, if you could send us an aliquot from your 129 Sv library. The institute my group and I are working at, the Max-Delbrück-Laboratory in the Max-Planck-Society, is a noncommercial research facility funded by the german government. It is understood that we will not distribute this library further without your consent.

Yours sincerely

Carmen Birchmeier

Dr. Carmen Birchmeier

Dr. Richard P. Harvey
The Walter and Eliza Hall Institute of Medical Research
Post Office, Royal Melbourne Hospital
Victoria 3050
AUSTRALIA
phone 61-3-3452485 facsimile 61-3-3470852

17.9.91

Dr. Anton Berns
Division of Molecular Genetics of the Netherlands Cancer Institute
Plesmanlaan 121
1066CX Amsterdam
Netherlands

Dear Anton,

It was good to bump into you again at the Wellcome gene targeting course in London. I have tried to get onto Stratagene about a 129 genomic library but that seems premature. I thought I would get in before the hoards and ask whether you could send me some of your library for our immediate needs. Of course, I understand if your stocks have been stretched by similar requests.

Yours sincerely and best wishes,

R. H.

Richard Harvey

Kosten berekenen

Kernforschungszentrum Karlsruhe

Kernforschungszentrum Karlsruhe GmbH Postfach 3640 W-7500 Karlsruhe 1

Dr. Anton Berns
Department of Virology
Antoni van Leeuwenhoekhuis
The Netherlands Cancer Institute
Plesmanlaan 121

NL-1066 CX Amsterdam
The Netherlands

Institut für Genetik und für
Toxikologie von Spaltstoffen

Leiter: Prof. Dr. P. Herrlich
Prof. Dr. D.M. Taylor

Datum: October 8. 1991/lk
Bearbeiter
Telefon: 07247/823292
Ihre Mitteilung:
FAX: 49 7247 82 3394

Dear Toni.

It was a pleasure to meet you and he influenced by your stimulating science orientation. As you had suggested we should first pull our gene out of the homologous ES cell library. Can we please use yours? We will meanwhile discuss the possibilities of knock-out constructs. I will then come back and ask for your advice if I may.

Thanks in advance.

regards.

yours

Peter Herrlich

Professor of Genetics
University of Karlsruhe
and
Director, Institute of Genetics and Toxicology
Kernforschungszentrum Karlsruhe

Dr. Anton Berns
Netherlands Cancer Institute
Plesmanlaan 121
1066 CX Amsterdam

Klaus Kästner
Institute for Cell and
Tumor Biology
DKFZ
Im Neuenheimer Feld
6900 Heidelberg

Heidelberg, 8-15-1991

Dear Dr. Berns,

during the recent "Wellcome Summer School on Gene Targeting and Homologous Recombination" in London you stressed the importance of the use of isogenic DNA for targeting experiments. I would greatly appreciate if you could supply us with an aliquot of an amplified genomic 129 or E14 library for our targeting experiments. Thank you in advance.

Sincerely

Klaus Kästner

Genentech, Inc.

460 Point San Bruno Boulevard
South San Francisco, CA 94080
(415) 266 1000
TWX: 9103717168

Dr. Anton Berns
The Netherlands Cancer Institute
Division of Molecular Genetics
Plesmanlaan 121
1066 CX Amsterdam
The Netherlands

Dear Dr. Berns:

I recently attended the Mouse Molecular Genetics Meeting in Heidelberg and noted with interest your results with isogenic DNA. I would be very interested in obtaining this 129 library from you.

I have recently begun working at Genentech were I would use this library. Please let me know what agreements or conditions would be needed.

If possible, please contact me by FAX (415-266 2739), or telephone collect (415-266 1984) with your response. I look forward to hearing from you.

Sincerely,

Mark W. Moore, Ph.D.

Dear Dr. Berns,

I would like to request the use of your 129 genomic library. I am a Senior member of the Department of Molecular Genetics at Hoffmann La-Roche and my lab is one floor above Andy McMahon's who has obtained your library. With your permission, I can get the library from him. Our aim is to create knockouts in the mouse V-cam and FI AM-1 genes. We are currently characterizing the mouse cDNAs for these genes and now need to pull out genomics. Actually we have ELAM genomics already from a BalbC library but would like to compare targeting frequencies with constructs from your 129 library.

I would greatly appreciate your help. We will not give out the library to anyone without your permission. If we can use your library please fax us a letter.

Sincerely,

Dr. Mark Labow
Department of Molecular Genetics
Hoffmann La-Roche Inc.
340 Kingsland St
Nutley, NJ 07110-1199

phone: 201-235-7073
Fax: 201-235-7617

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EXHIBIT L

Howard Hughes Medical Institute
Research Laboratories / Seattle

University of Washington School of Medicine
Mail Stop SL-15
Seattle, Washington 98195

Richard D. Palmiter, Ph.D.
Investigator
Telephone (206) 543-6090

3 September 1991

Dr. Anton Berns
Netherlands Cancer Institute
Amsterdam

Dear Anton,

It was good to see you again. I want to thank you for being chairperson once again. It was a very good session and the last talk was especially good! I was also glad to hear that you will become a co-organizer in future years. I feel assured that the meeting is in good hands. It is really not very much work, but after four years I think it is good to get some fresh input.

I wanted to follow up on the observations that Hein te Riele discussed regarding the importance of using strain 129 DNA for homologous recombination in ES cells. We have been trying to target the dopamine beta-hydorxylase gene with great difficulty (about 1/800 neo^R cells are targeted) and would like to isolate the gene from your 129 lambda library, if necessary. We would certainly use it for all new gene isolations as well since it cannot hurt. Thus, if you would be willing to send an aliquot of your 129 lambda library I would be most grateful.

If you use Federal Express you can bill it to me by using the following numbers:

Under payment: check the box, "bill third party" and enter 1253-3198-0

Under billing reference: enter 027-756

The Federal express address is: Howard Hughes Med Inst, Univ of WA Health Sci Bldg I 605, Seattle WA 98195
Phone (206) 543-6064

Thank you very much.

Sincerely,

Richard Palmiter

UNIVERSITE LIBRE DE BRUXELLES

ULB

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Dr Hein Te Riele
Division of Molecular Genetics
The Netherlands Cancer Institute
Plesmanlaan 121
1066 CX Amsterdam
The Netherlands

October 1, 1991.

Dear Dr Te Riele,

As a follow up of our phone conversation, I would be interested in obtaining your genomic library derived from mouse strain 129. We are primarily interested in the development of homologous recombination in ES cells using genes of the G protein coupled family of receptors. We are ready to pay all charges related to the shipment of this material and will not distribute the library without your prior consent. In case you send the package via Fodoral Express, you can charge our account number 1360-1622-9.

I thank you for your kind consideration, and I remain,

Sincerely yours,

Parmentier

M. Parmentier

I.R.I.B.H.N.
ULB Campus Erasme
Building C 5th floor, room 135
808 route de Lennik
B-1070 Brussels Belgium
Phone 32-2-555 41 72
Fax 32-2-555 46 55

The Jackson Laboratory
Bar Harbor, Maine 04609
(207) 288-3371

Jeffrey D. Saffer
Research Scientist

December 13, 1991

Dr. Anton Berns
Department of Molecular Genetics
The Netherlands Cancer Institute
Plesmanlaan 121
1066 CX Amsterdam
The Netherlands

Dear Dr. Berns:

I am writing for two reasons. The first is to thank you and Dr. te Riele for sending the retinoblastoma targeting vectors and probes. As Ken Paigen probably described, we are setting up a gene targeting lab. We had tried some preliminary experiments with a targeting vector for the uncoupling protein gene without success. We appreciate your sharing your clones with us so that we could demonstrate that we could carry out homologous recombination with good vectors. In accordance with your results, we have been successful getting fairly efficient homologous recombination with your clone. This exercise has been most useful for us in working out the methods.

Second, given the potential benefits of using 129-derived clones in the targeting vectors, I would like to get your 129 lambda genomic library. We would appreciate this greatly.

Thanks again for your help.

Sincerely,

Jeffrey D. Saffer

P.S. Ken says "Hi and thanks".

cc. Hein

MAX-DELBRÜCK-LABORATORIUM
in der Max-Planck-Gesellschaft

Carl-von-Linné-Weg 10. D-5000 Köln 30. Germany. Tel.: 49-221-5062 615. Fax: 49-221-5062 613
Dr. Silvia Stabel

Dr. Anton Berns
Division of Molecular Genetics
Netherlands Cancer Institute
Plesmanlaan 121
NL - 1066 CX Amsterdam

21st September 1992

Dear Dr. Berns,
with interest I read your paper which appeared in June this year in PNAS (te Riele et al.).
Together with Achim Gossler here in the institute we have been trying to target the protein kinase C-γ gene in the D-3 cell line with non-isogenic DNA and have not been successful so far.
Apart from other factors which might affect the targeting frequency and which we also try to change we would also like to use isogenic DNA for our next attempt. Therefore I would like to ask you, if you would make available your 129 genomic DNA library for our targeting project.
I thank you very much in advance if you can help us in this matter.
With best regards

Silvia Stabel

Dr. Silvia Stabel

cc. Heinz 24/9

received 30/07/92

Dennis

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D-5000 Köln 41
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20 July 1992

Dear Dr. te Riele,

I am a post-doctoral fellow working in Klaus Rajewsky's laboratory at the Institute for Genetics in Cologne and am writing to ask if it would be possible to obtain the 129-derived genomic library which you have used successfully? Thank-you very much in advance.

Sincerely,

Raul M. Torres Ph.D.

Institute for Genetics
University of Cologne
121 Weyertal
D-5000 Cologne 51
Germany

phone: (49 221) 470 34 16
fax: (49 221) 470 5185

cc: → Dennis
23/7/92